PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 04 May 2001 (04.05.01)	
International application No. PCT/EP00/07102	**Applicant's or agent's file reference** SCB577PCT
International filing date (day/month/year) 25 July 2000 (25.07.00)	**Priority date** (day/month/year) 26 July 1999 (26.07.99)
Applicant MORRONE, Raffaele et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 February 2001 (08.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SCB577PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/07102	25/07/2000	26/07/1999
International Patent Classification (IPC) or national classification and IPC C12P41/00		
Applicant CONSIGLIO NAZIONALE DELLE RICERCHE		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/02/2001	10.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Lopez Garcia, F Telephone No. +49 89 2399 2171

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/07102

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-7 as originally filed

Claims, No.:

1-9 as originally filed

Drawings, sheets:

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application.

The following defects in the form or contents of the international application have been noted:
see separate sheet

Re lt m V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive st p or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

   D1: EP-A-0 510 712
   D2: EP-A-0 407 033
   D3: US-A-4 107 439

2. The application describes the use of orthoesters in the synthesis of chiral acids in biocatalyzed esterification processes in order to remove water.

3. D1 (examples) and D2 (see for instance example 3) describe biocatalyzed esterification processes for the synthesis of chiral acids. They do not use any orthoester.

4. D3 describes chemical esterification processes for the preparation of esters where an orthoester is used in order to remove water (see col. 9, l. 6-12).

5. Novelty is acknowledged for the subject-matter of claims 1-9 (Art. 33(2) PCT).

6. D1 and D2 are considered the closest prior art.

   The technical problem seems to be the provision of further methods for the resolution of enantiomeric mixtures of a chiral carboxylic acid.

   The solution proposed, which consists in the addition of orthoesters to the reaction in order to make the process irreversible by removal of water, appears to be not inventive.

   The subject-matter, which concerns to esterification reactions, is well known in the art (D1, D2 and p. 1, l. 3 of the application). The limitations due the reversibility of the esterification reaction are also known (see p. 1, l. 8-27 of the application). In order to overcome said limitations, many approaches have been proposed (see p.

2, l.1-21 of the application) which mainly consist in the removal of water from the reaction. Therefore, the skilled person, when faced with the problem of providing further method for the resolution of enantiomeric mixtures of a chiral carboxylic acid, would also think in the possibility of removing water by additional methods. D3 discloses that the water present in esterification processes can be removed by addition of orthoester (see col. 9, l. 6-12). Therefore, the use of orthoesters appears as an obvious alternative to those already mentioned in the application (see p. 2, l.1-21 of the application) and not inventive merit can be recognized in absence of any unexpected/surprising effect.

Therefore, the subject-matter of claims 1-9 is not inventive (Art. 33(3) PCT).

Re Item VII

Certain defects in the international application

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D3 is not mentioned in the description, nor are these documents identified therein.

2. No support is found in the description found for the subject-matter of claim 6, ie the esterification process of the meso form of a bicarboxylic acid.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant *(for all designated States except US)*: CONSIGLIO NAZIONALE DELLE RICERCHE [IT/IT]; Piazzale Aldo Moro, 7, I-00185 Roma (IT).

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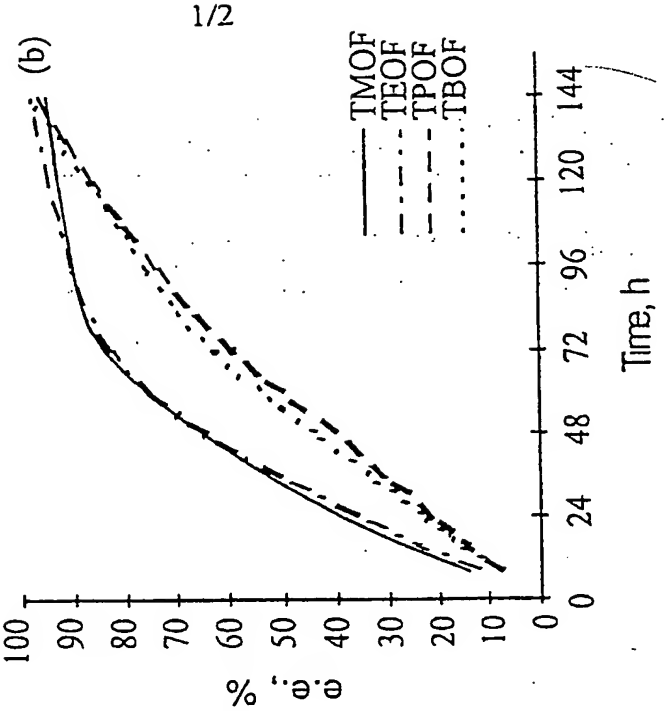
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/07564 A2

(54) Title: THE USE OF ORTHOESTERS FOR THE SYNTHESIS OF CHIRAL ACIDS IN BIOCATALYZED IRREVERSIBLE ESTERIFICATION PROCESSES

(57) Abstract: A process for the resolution of enantiomeric mixtures of a chiral carboxylic acid, comprising an esterification reaction of said carboxylic acid in an organic solvent, in the presence of a stereoselective hydrolase, characterized in that an orthoester of the formula: R^1-$C(OR^2)_3$, in which R^1 is selected from H and C_1-C_4alkyl and R^2 is C_1-C_8alkyl or -CH_2-C_{6-10}aryl, is used as the esterification reactive.



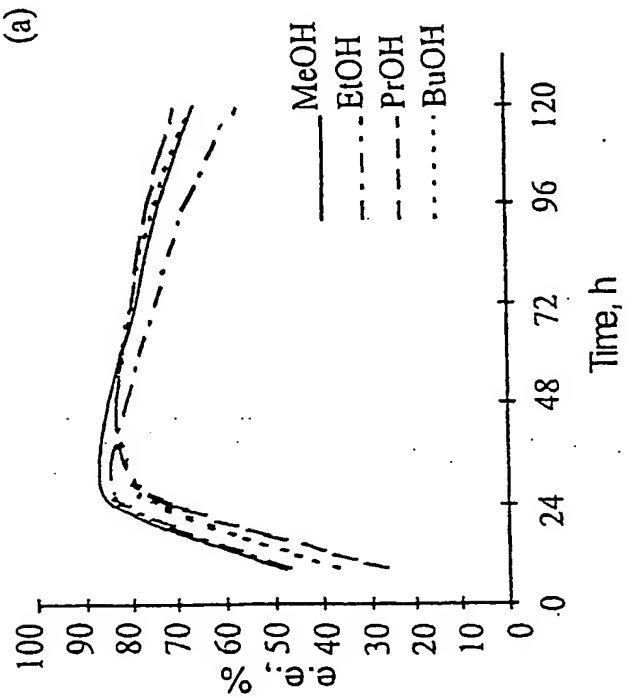


Fig. 1. Enantiomeric excess (ee) value of unreacted Flurbiprofen versus reaction time with different alcohols (a) and orthoformates (b)

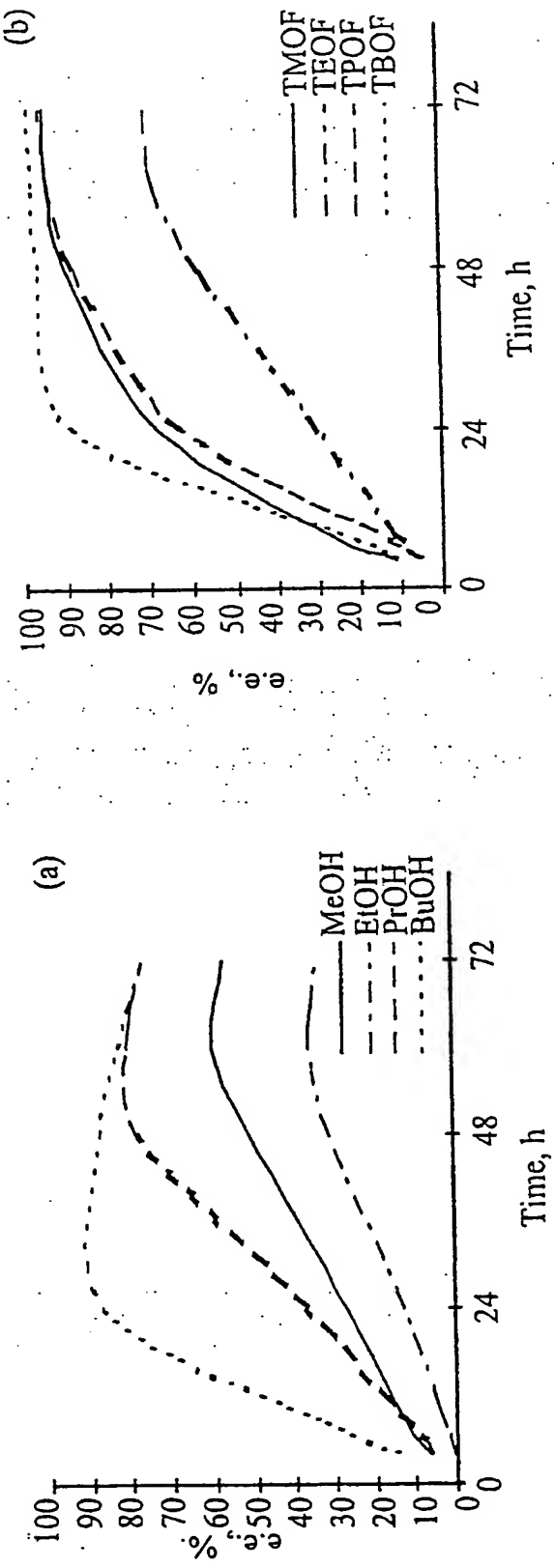


Fig. 2. Enantiomeric excess (ee) value of unreacted 2-Methylvaleric acid versus reaction time with different alcohols (a) and orthoformates (b)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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WO 01/07564 A3

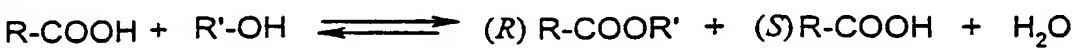
(54) Title: THE USE OF ORTHOESTERS FOR THE SYNTHESIS OF CHIRAL ACIDS IN BIOCATALYZED IRREVERSIBLE ESTERIFICATION PROCESSES

(57) Abstract: A process for the resolution of enantiomeric mixtures of a chiral carboxylic acid, comprising an esterification reaction of said carboxylic acid in an organic solvent, in the presence of a stereoselective hydrolase, characterized in that an orthoester of the formula: R^1-$C(OR^2)_3$, in which R^1 is selected from H and C_1-C_4alkyl and R^2 is C_1-C_8alkyl or -CH_2-C_{6-10}aryl, is used as the esterification reactive.

THE USE OF ORTHOESTERS FOR THE SYNTHESIS OF CHIRAL ACIDS IN BIOCATALYZED IRREVERSIBLE ESTERIFICATION PROCESSES

Enantiomerically pure chiral compounds are increasingly required in recent times, as these compounds may be used in a number of different fields (biomedical, agroalimentary, special materials and the like). Racemic chiral acids may be resolved by means of esterification in organic solvent, catalyzed by a hydrolase (lipase, esterase, protease), as illustrated for example in IT 1 274 482 and IT 1 275458.

When a racemic acid RCOOH is reacted with an alcohol R'OH in the presence of a hydrolase with R-stereopreference, this enantiomer will be the fast reacting one, undergoing more rapidly the esterification, so that the unreacted acid will enrich in the S enantiomer, according to the following scheme:



Apparently, it seems possible to obtain the optically pure S isomer simply by extending the conversion to a sufficiently high value. However the reversibility of this reaction makes the situation complicated, as the R enantiomer, which is the faster formed one, is also the one more easily undergoing hydrolysis, to the detriment of the optical purities of both the R ester and the S acid residue (Chen, C. S.; Wu, S. H.; Girdaukas, G. and SiH, C. *J. Am. Chem. Soc.* **1987**, *109*, 2812 – 2817).

The above mentioned limits are also found in the desymmetrization of polycarboxylic acids meso-forms, when carrying out their enantiotoposelective esterification in the presence of hydrolase.

Many approaches have been proposed to overcome the problems connected with the reversibility of the esterification reaction:

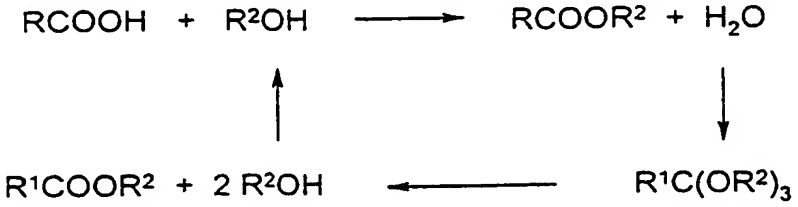
a) Removing water from the reaction equilibrium by addition of dehydrating salts (Kvittingen, L.; Sjursnes, B. and Anthonsen, T. *Tetrahedron* **1992**, *48*, 2793-2802). The drawback of the process is that the collisions between the salt particles and the enzyme ones damage the latter, thus reducing the life times and making their recovery difficult.

b) Removing water from the equilibrium by addition of molecular sieves (Fonteyn, F.; Blecker, C.; Lognay, G.; Marlier, M. and Severin, M. *Biotechnol. Lett.* **1994**, *16*, 693-696). In addition to the above drawbacks, the alcohol also can be removed, particularly in case of low molecular alcohols.

c) Removing water by distillation. This method can be used only when water is the lower boiling component of the mixture; therefore it cannot be used with low boiling alcohols or solvents.

d) Recycle of the reaction products to increase their optical purity (Morrone, R.; Nicolosi, G.; Patti, A. and Piattelli, M. *Tetrahedron: Asymmetry* **1995**, *6*, 1773-1778). This method clearly increases the work up costs.

It has now been found, and this is the object of the invention, that when the reaction is carried out in the presence of orthoesters, the latter react with water formed during the reaction, making therefore the process irreversible.



DISCLOSURE OF THE INVENTION

The present invention therefore provides a process for the resolution of enantiomeric mixtures of a chiral carboxylic acid of formula

$$R\text{-}COOH,$$

wherein R is a hydrocarbon residue optionally containing one or more heteroatoms and optionally mono- or polysubstituted, comprising an esterification reaction of said carboxylic acid in an organic solvent, in the presence of a stereoselective hydrolase, characterized in that an orthoester of formula

$$R^1\text{-}C(OR^2)_3,$$

in which R^1 is selected from H and C_1-C_4alkyl and R^2 is C_1-C_8alkyl or -CH_2-C_{6-10}aryl,

is used as the esterification reactive.

R is preferably the residue of an antiinflammatory arylpropionic acid such as (±)-(*R,S*)-2-(2-fluoro-4-biphenyl)-propionic, (±)-(*R,S*)-2-(3-benzoylphenyl)-propionic, (±)-(*R,S*)-2-(4-isobutylphenyl)-propionic, (±)-(*R,S*)-2-[4-(1-oxo-2-isoindolinyl)phenyl]propionic, (±)-(*R,S*)-2-[4-(2-thenoyl)phenyl]-propionic, (±)-(*R,S*)-2-(6-methoxy-2-naphthyl)-propionic acids.

R^1 is preferably selected from H, methyl, ethyl, n-propyl, n-butyl.

The stereoselective hydrolase is preferably a lipase from Candida antarctica, Candida cylindracea, Pseudomonas cepacia, Mucor miehei, Mucor javanicus, Aspergillus niger, swine pancreas, or a protease from Aspergillus subtilis.

The esterification reaction is generally carried out at a temperature of 0-50°C, preferably at 45°C. Similarly, a supercritical gas, such as CO_2, can be used as the reaction solvent.

Conveniently the process according to the invention comprises the step of adding to the reaction mixture, consisting of the carboxylic acid, the hydrolase and the organic solvent, an amount of water or of a alcohol with 1-8 carbon atoms equivalent to 1-5% mols compared with the mols of said chiral carboxylic acid. The reaction is thereby activated, which then proceeds thanks to the formation of the alcohol following reaction of the orthoester with the water formed during the esterification reaction.

The resulting suspension is kept under stirring at the optimal temperature for the enzyme used. The progress of the reaction can be monitored by the usual analytical methods known to those skilled in the art. When the desired conversion value, on which the desired enantiomeric excess of the products depends, has been reached, the reaction is stopped by filtering off the enzyme. The reaction products are then recovered by separation with procedures known to those skilled in the art.

Alternatively to the use of orthoesters, carbonates may also be used in the process of the invention.

The irreversibility of the esterification, carried out with the process of the invention, allows to prepare chiral acids in enantiopure form (in particular the enantiomer not preferred by the enzyme) by extending the reaction times up to conversion values higher than 50%.

Figure 1a shows the change of the optical purity of the unreacted substrate in the esterification of rac-flurbiprofen, depending on the reaction time, when using methanol, ethanol, propanol and butanol as alcohol, acetonitrile as solvent and a lipase from Candida antarctica (with R stereopreference). In Figure 1b it is reported the progress of the reaction, under the same operative

conditions, using orthoformate (respectively methyl, ethyl, propyl, butyl) as alcohol source.

When comparing the progress of the reaction with alcohols (Figure 1a) and that with orthoformates (figure 1b) it is easily evident that in normal esterification of flurbiprofen the ee of the unchanged substrate reaches a maximum value of 80-85 and then begins to drop.

In patent contrast, when orthoformates are used the ee value continues to increase by extending the incubation period and consequently the conversion value. With all the orthoformates tested, the ee value of the unreacted acid reaches 95-98%.

In Figure 2 it is reported the trend for the esterification in hexane of 2-methylvaleric acid in the presence of Candida cylindracea lipase (Stereopreference S). The esterification with alcohol (Figure 2a) shows the usual course of the reversible reactions and the ee of the residual acid decreased when conversion is extended much beyond 50%. The esterification with the use of orthoformates proceeded as an irreversible reaction (Figure 2b) and with the best of the four tested, tributyl orthoformate, the ee values of the remaining substrate obtained is >98.

Obviously, the method proposed here can be used not only in the resolution of chiral acids, but also in the esterification of achiral acids, particularly when they are very expensive, to increase the yield by pushing the equilibrium toward completion.

The following examples disclose the invention in more detail.

Example 1

Preparation of enantiopure S-flurbiprofen

Novozym 435(R) (lipase from Candida antartica) (100 g) was added to a solution of racemic flurbiprofen (41 mmol, 10 g) in CH_3CN (1 l) containing tripropyl orthoformate (123 mmol, 26.5 ml) and 0.1 ml of n-propanol. The mixture was incubated at 45°C under shaking (300 rpm) and conversion and ee of unreacted flurbiprofen were followed by hplc using a Chirex R-NGLY & DNB (250 x 4.0 mm) column. After 6 days conversion had reached 60% and the reaction was stopped filtering off the enzyme. Removal of the solvent in vacuo left a residue that was partitioned between hexane and aq. $NaHCO_3$ (3 g in 200 ml of water). The organic phase was washed with water, dried over Na_2SO_4 and the solvent removed to afford 6.8 g of (-)-R-flurbiprofen propyl ester (yield 58%, ee 64%). ^1H NMR ($CDCl_3$): δ 0.89 (t, 3H, J=7Hz), 1.54 (d, 3H, J=7Hz), 1.65 (m,2H), 3.78 (q, 1H, J=7Hz), 4.06 (t, 2H, J=6Hz), 7.1-7.6 (m, 8H). Anal. Calcd for $C_{18}H_{19}FO_2$; C, 75.70; H, 6.69. Found: C. 75.62; H, 6.89.

Acidification of the aqueous phase with H_2SO_4 gave a precipitate of (+)-S-flurbiprofen (3.9 g, yield 39%, ee>98%). Anal. Calcd for $C_{15}H_{13}FO_2$; C, 73.76; H, 5.36. Found: C. 73.90; H, 5.52.

Example 2

Preparation of enantiopure (R)-2-Methylvaleric acid

Candida cylindracea lipase (50 g) was added to a solution of racemic 2-methylvaleric acid (86.2 mmol, 10 g) in hexane (500 ml) containing tributyl orthoformate (86.2 mmol, 23 ml) and 0.1 ml of n-butanol. The mixture was incubated at 45°C under shaking (300 rpm). Conversion and ee of the butyl ester were followed by GC using a ß-cyclodextrin (dimethylpenthylbetacdx/OV1701 3:7) column. After 48 h conversion had reached 65% and reaction was

stopped filtering off the enzyme. After partition with aq. $NaHCO_3$ (3 g in 200 ml of water) the hexane phase was dried over Na_2SO_4 and evaporated under vacuum to furnish 9.6 g of (S)-2-methylvaleric butyl ester (yield 65%, ee 53%). MS data agreed with those reported in the literature (Kim Ha, J.; Lindsay, R.C.; J. Food Compos. Anal. 1989, 2, 118-131). Anal. Calcd for $C_{10}H_{20}O_2$; C, 69.72; H, 11.70. Found: C. 69.98; H, 11.84.

The aqueous phase was acidified with H_2SO_4, extracted three times with hexane and the organic phase were pooled. Removing of hexane under vacuum gave 3.5 g of (R)-2-methylvaleric acid (yield 35%, ee>97%). $[a]_D^{20}$= 18.2 (neat); (lit. $[a]_D^{20}$= 18.4 (neat); Levene, P. A.; Marker, R. E. J. Biol. Chem. 1932, 98,1) Anal. Calcd for $C_6H_{12}O_2$; C, 62.04; H, 10.41. Found: C. 62.31; H, 10.52.

CLAIMS

1. A process for the resolution of enantiomeric mixtures of a chiral carboxylic acid of formula

R-COOH,

wherein R is a hydrocarbon residue optionally containing one or more heteroatoms and optionally mono- or polysubstituted, comprising an esterification reaction of said carboxylic acid in an organic solvent, in the presence of a stereoselective hydrolase, characterized in that an orthoester of formula

R^1-$C(OR^2)_3$,

in which R^1 is selected from H and C_1-C_4alkyl and R^2 is C_1-C_8alkyl or -CH_2-C_{6-10}aryl,

is used as the esterification reactive.

2. A process as claimed in claim 1, wherein R^1 is selected from H, methyl, ethyl, *n*-propyl, *n*-butyl.

3. A process as claimed in claim 2, wherein said stereoselective hydrolase is a lipase selected from Candida antarctica, Candida cylindracea, Pseudomonas cepacia, Mucor miehei, Mucor javanicus, Aspergillus niger, swine pancreas, or a protease from Aspergillus subtilis.

4. A process as claimed in any one of the above claims, wherein said esterification reaction is carried out at a temperature of 0-50°C, preferably at 45°C.

5. A process as claimed in any one of the above claims comprising the step of adding the reaction mixture with an amount of water or of a alcohol with 1-8 carbon atoms equivalent to 1-5% mols compared with the mols of said chiral carboxylic acid.

6. A process as claimed in any one of the above claims, wherein in said esterification reaction the meso form of a bicarboxylic acid is used as substrate.

7. A process as claimed in the above claims 1-6, wherein said carboxylic acid is selected from (±)-(*R,S*)-2-(2-fluoro-4-biphenyl)-propionic, (±)-(*R,S*)-2-(3-benzoylphenyl)-propionic, (±)-(*R,S*)-2-(4-isobutylphenyl)-propionic, (±)-(*R,S*)-2-[4-(1-oxo-2-isoindolinyl)phenyl]propionic, (±)-(*R,S*)-2-[4-(2-thenoyl)phenyl]-propionic, (±)-(*R,S*)-2-(6-methoxy-2-naphthyl)-propionic acids.

8. The use of an orthoester of formula

$$R^1\text{-}C(OR^2)_3,$$

in which R^1 is selected from H and C_1-C_4alkyl and R^2 is C_1-C_8alkyl or -CH_2-C_{6-10}aryl,
in combination with a stereoselective hydrolase in the resolution of enantiomeric mixtures of carboxylic chiral acids.

9. The use as claimed in claim 8, wherein said hydrolase is a lipase selected from Candida antarctica, Candida cylindracea, Pseudomonas cepacia, Mucor miehei, Mucor javanicus, Aspergillus niger, swine pancreas, or a protease from Aspergillus subtilis.

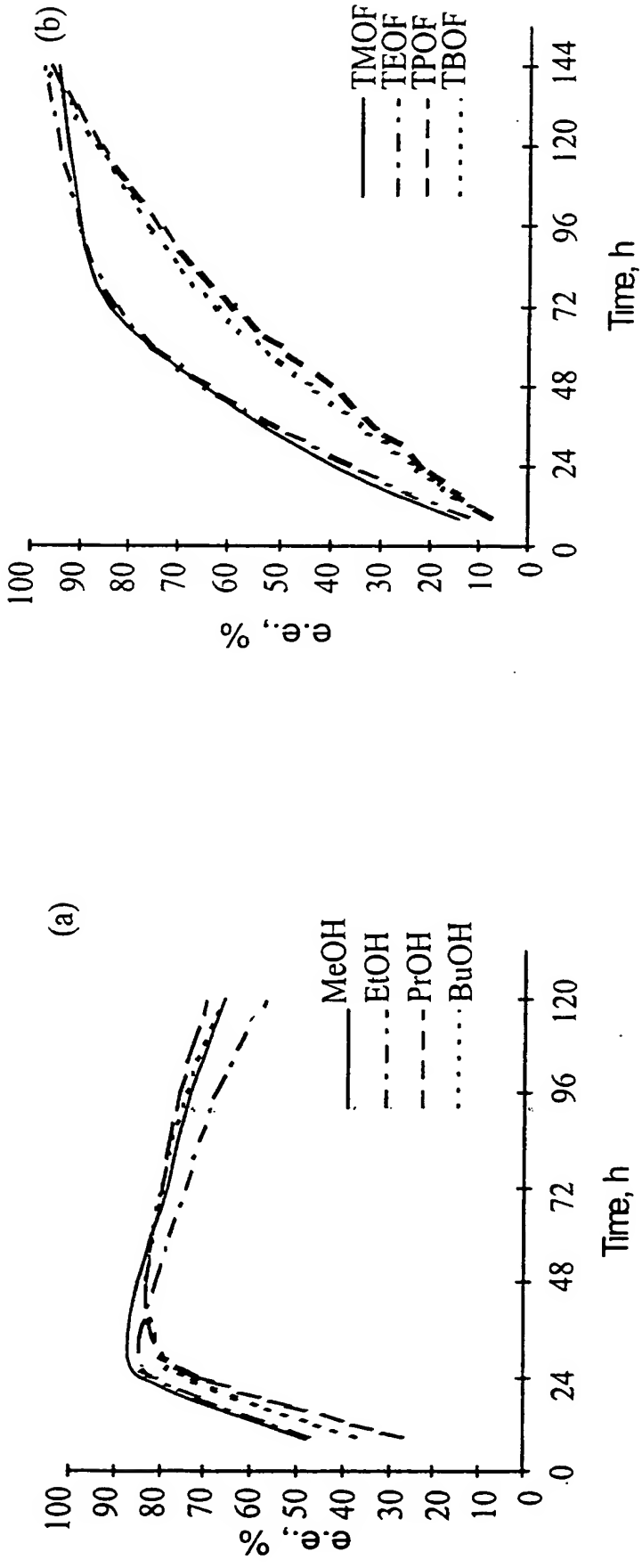
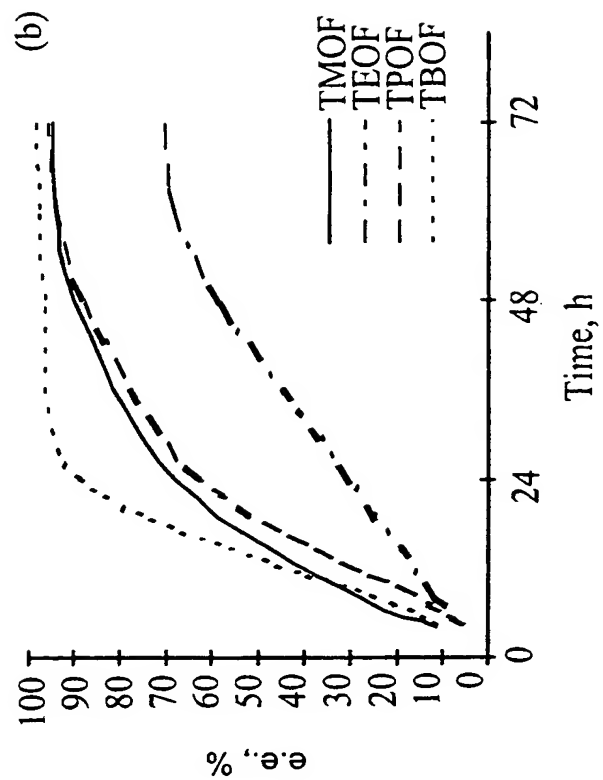


Fig. 1. Enantiomeric excess (ee) value of unreacted Flurbiprofen versus reaction time with different alcohols (a) and orthoformates (b)



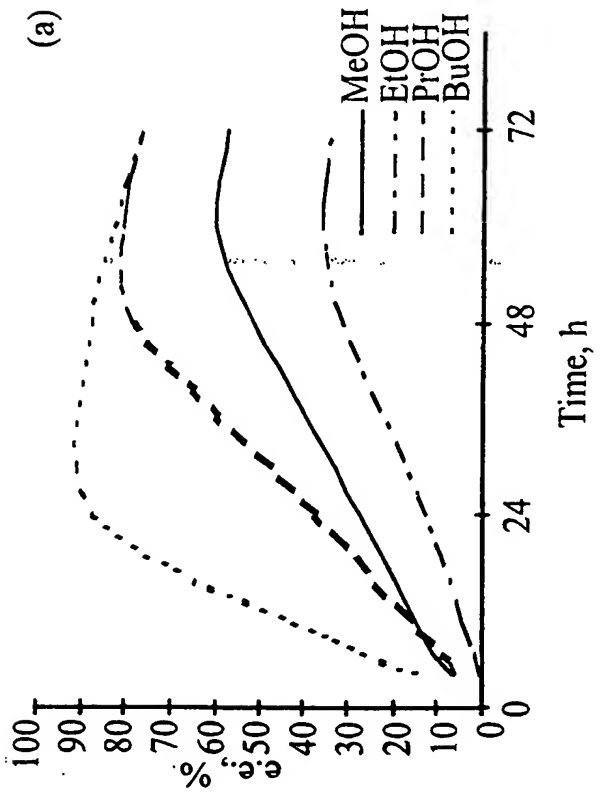


Fig. 2. Enantiomeric excess (ee) value of unreacted 2-Methylvaleric acid versus reaction time with different alcohols (a) and orthoformates (b)

NTERNATIONAL SEARCH REPOR

Interna al Application No
PCT/EP 00/07102

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12P41/00 C12P7/40 C12N9/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12P C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 510 712 A (MINI RICERCA SCIENT TECNOLOG) 28 October 1992 (1992-10-28) claims 1-7; examples 1-11	1-9
A	EP 0 407 033 A (RHONE POULENC IND) 9 January 1991 (1991-01-09) claims 1,2,11-36	1-9
A	US 4 107 439 A (WALKER JERRY A ET AL) 15 August 1978 (1978-08-15) column 8, line 63 -column 9, line 12	1-9

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
18 January 2001	25/01/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van Klompenburg, W

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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040. Tx. 31 651 epo nl. Fax: (+31–70) 340–3016	van Klompenburg, W

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